

Gene	Upstream Primer	Downstream Primer	Use	Source
<i>cyoA</i>	cyoA-XbaI <u>GATCTAGAAGCGTCCGGTCAGCCT</u>	cyoA-HndIII GTAAGCTTCGGCGGTGTCATCCAG	Mutagenesis	Lunak & Noel, 2015
<i>ActS</i> - upstream	ActSup-EcoRI <u>GCGAATTCA</u> GGAGGTACTGCAGCTT	ActSup-XbaI GTT <u>CAGA</u> TAGCGATCAGCGGCAAC	Mutagenesis	This work
<i>ActR</i> -downstream	ActRdwn-XbaI <u>GCTCTAGA</u> CACGCCGGCTCAATATG	ActRdwn-HndIII GTAAGCTT <u>GCGAGGA</u> ATCCGTCTGC	Mutagenesis	This work
<i>ActSR</i>	ActSup-EcoRI <u>GCGAATTCA</u> GGAGGTACTGCAGCTT	ActRdwn-HndIII GTAAGCTT <u>GCGAGGA</u> ATCCGTCTGC	Complementation	This work
<i>cyoA</i>	cyoA-BamHI <u>GCGGATCCC</u> GGTTCAAGCGACGACGA	cyoA-PstI TGCT <u>GCAGTT</u> CAAGGAGGTCAAGGATT	Complementation	Lunak & Noel, 2015
PcyoA	PCyoA-KpnI <u>GCGGTACCTT</u> CAAGCGACGACGA	PcyoA-XbaI GAT <u>CTAGAC</u> AGCGGCAAGACGGATA	<i>cyoA::lacZ</i> fusion	This work
<i>cyoB</i>	cyoBRT-Forward TGTTCGGCTATGCCTCAATG	cyoBRT-Reverse CCGAAGAAGGAATTGACGCT	RT-qPCR	Lunak & Noel, 2015
16sRNA	16sRNART-Forward TGGAGTATGGAAGAGGTGAG	16sRNART-Reverse TCAGTAATGGACCAGTGAGC	RT-qPCR	Lunak & Noel, 2015

Table 1: Primers used in study.

A) *R. etli* CFN42

TAAGGCGCTTGCGGCAAGGCGCCTCAAGTCTGATCTTGATCAAACCTGCAGGCTTATCAAGTCCGTAAGACCGAAAT
AGTCATCATCACGGCTCAAATGAAGCGCAGATGGAAAAAATTGCGTGATCCGGACGGTGATTTCCCCTGCAAATG
CTGGCCTGACCGGCAGTTGCGGATATATTGCACTGAACATGCGCAGGGCTAAATCCTTCTATATTCCCATCAGGAT
TTCAAAGCACTAGCGCCCGATGTTTGCGGACAAACACAAGAGCTAGAGAC**GTC**CCAAACTC**ATG**AAGTTT
TATCCGGTTCAGTGCTTAGTGCGGACAAACACAAGAGCTAGAGAC**GTC**CCAAACTC**ATG**AAGTTT
CCCGCTTCTATCCGTCTTGCCGTGTTCCCTGGCAGGATGCAAAC**ATG**TGGTCATGGCGC

B) *S. meliloti* 1021

TGTCGTCGTGAATGCCCCGCGCTCCATCGAAGAGGACCGGTCCAGGACTGTTGCATTGCACTGCTC
ATCAAACGCCTCCCGCGCTCGACGGGGCTAAGAACATGCGGTTTCCCGCCACCCGCTCGACTCTCAAGAGTGA
GAGCGCAAGGCTCCCTGGAGGGATCTTGACCAATCAACGTTCATATCAAGGGAGAAACCGGAAAATAATGCTTT
CACGTCCTCACTGTCTGCAAGCGGGAAGATGCTGCGTCGAAACCGACTGCGGCGATTGCCTGCAGTGCGAC
CGACCTCATACCTTCCATCCACTCTACTTTAGTCGCAGGGCCGTCGAAACAAAATAGGTCCGAGCCGGTCCAG
AACTGTGAAGTTCCCGCCGCCTCGCCGTTTGCCCGTTTCCCTGTATGGCGGGATGCGACA**AT**GGTG

C) *R. leguminosarum* bv *viciae* 3841

GTAGCCATGAAACGCCTCCCTCGTTGAGGCGCTTCGCAAGGCCTCAACCTGATCTTGATCAAACCTGCAGGCT
TATCAAGTCCGTAAGACGAAAATAGTCATATCACGGCTAAATGAGGCGTAACGGAAAAATAGCGGTGACCG
GAGCGCGATTTCCGGGGACGTCGCTGGCTGGCCGGCCTTGCGGATATATGGAAACAAACATGCCTGGCGAAAG
AATCCTCTGTATTCCAGGATCCAAAGCTGTAGTCACCCATCTGTGCCGAG**T**GCGGCA**T**GTGCGT
CTGCGACCAAACACCTCATGTCGCTATCGGGTTCAGTCGTTAGTCCGGAACGAACAAACACAAGAGCTTAGAG
AC**GTGCTAAAACCTGGGAAGTTCCGCCTCTATCGGTCTGCGTCTGGCAGGATGCAAAC**AT**GGTG**

D) *B. japonicum* USDA 110

GGCGGGAAACACGACGATACCGAGGGCGATCGGTAGACGAAGGAAGTCGAAGAATTCCGAGGG**T**GCGGCCGATGTGACG
CGATGGCGATCTCGCCGGACGTGGCTGGCTGGCCGTCTGCCCAGGTGGAGGTCTGCCCATTCAGGGGTCT
GCCGTCGCCATTC**T**CGCCCTTAACGTCTGCAAACAAAGCCACCCGGCCGGCGCCAGGAATCCGGCC
TGTCTGGCCAAACATCCCGACTCGAACATGGACAAATGTCCAAATGTCCGGATGTCGCCCGAC**G**CTACCCGT
GGCCCCCGAAAGAAACCTATCTCAAGGGCTGGCCCG**T**GTCCCGTCTCAAGATCTGGCGCTGTACCCTTGGCA

E)	Consensus	NGNGC NNNNNNNN
R. etli 1	TGCGGCAAGGCGCC	
R. etli 2	TGCGGC AT <u>GT</u> TT <u>GC</u> T C	
R. etli 3	TGCGAC CT <u>CC</u> GC <u>CC</u>	
S. meliloti 1	TGCGCC CG <u>CG</u> GT <u>CG</u>	
S. meliloti 2	TGCGGCGG AT <u>T</u> <u>GC</u> T <u>GC</u>	
S. meliloti 3	AGCGGCGG AA <u>GA</u> GT <u>GC</u>	
S. meliloti 4	TGCGAC CC <u>AT</u> <u>CG</u> AC <u>CT</u>	
R. leguminosarum 1	TGCGGC AT <u>G</u> TT <u>GC</u> T C	
R. leguminosarum 2	TGCGAC CA <u>AA</u> AC <u>AC</u> CT <u>C</u>	
B. japonicum 1	TGCGGCC GT <u>AT</u> <u>GT</u> GA <u>CG</u>	
B. japonicum 2	TGCGCC CT <u>TAAC</u> <u>GT</u> CC	
B. japonicum 3	TGCGCG CA <u>G</u> CT <u>AC</u>	

Figure S1 Nucleotide sequences 5' of potential *cyoA* orfs in **(A)** *Rhizobium etli* CFN42 (nucleotides 40,430 – 39,982) **(B)** *S. meliloti* 1021 (nucleotides 1407913-1408369) **(C)** *R. leguminosarum* bv *viciae* (nucleotides 53,510-53,971) and **(D)** *Bradyrhizobium japonicum* USDA 110 (nucleotides 144990-145374). Putative ActR DNA binding sites are underlined (_). A putative CRP-FNR anaerobox is dashed-underline (__). Possible ATG translation start sites are highlighted in black. **(E)** Listed potential ActR DNA binding sites. In bold are the conserved residues of the consensus ActR DNA binding sites from *B. japonicum* (Lindemann *et al.*, 2007; Torres *et al.*, 2014; Emmerich *et al.*, 2000).

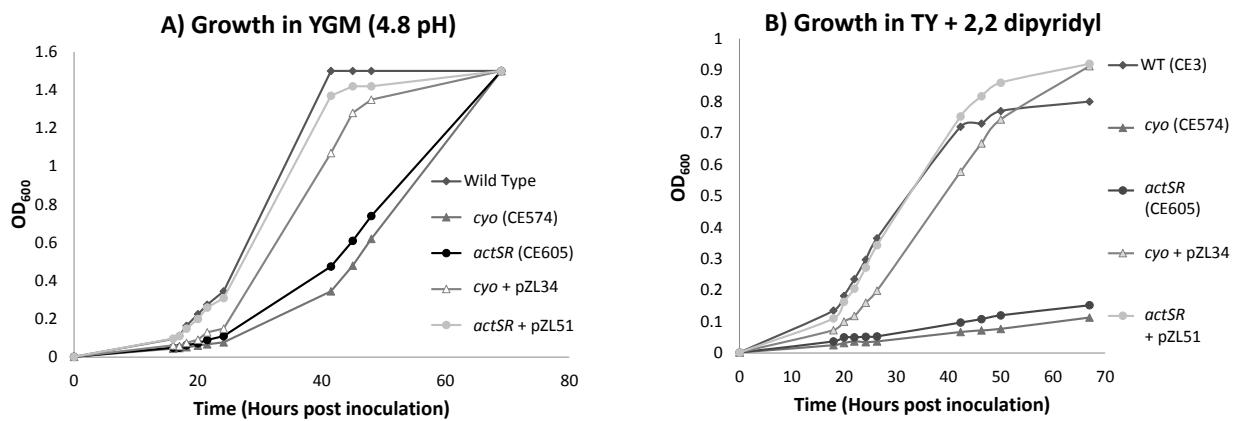


Figure S2: Complementation of *cyo* and *actSR* mutants at low pH (A) and low iron (B).

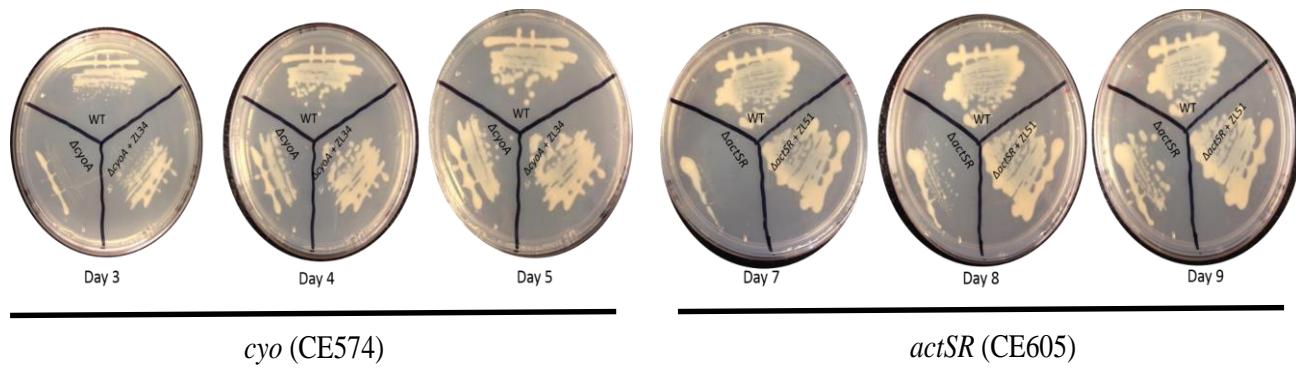


Figure S3: Growth on low pH YGM plates. Colonies were picked from TY plates and streaked on YGM agar buffered with MES at 4.8 pH. At least three different colonies for each strain were tested. Below the plates indicates the number of days the plate was incubated. The strain that was streaked is indicated in the triangle on the plates. The upper triangle is the wild type, lower left triangle is the mutant (*actSR* or *cyo*), and lower right is the mutant carrying the complemented plasmid (pZL34 or pZL51).